J. Trop. Plant Pests Dis.
Vol. 23, No. 2, September 2023
Pages: 65–70

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.12365-70

RESEARCH PAPER

The role of bacterial consortium as bioactivator to stimulate production and suppress grain rot disease and bacterial leaf blight in rice

Fadyah Khamila Sahlan, Nur Fadhillah Ayu Eka, Muhammad Junaid, Nur Hardina, Nurmujahidin, Tutik Kuswinanti, & Baharuddin Patandjengi

Manuscript received: 25 January 2023. Revision accepted: 17 May 2023. Available online: 28 August 2023.

ABSTRACT

Burkholderia glumae and *Xanthomonas oryzae* pv. *oryzae* are the primary causes of bacterial disease in rice, capable of reducing production and posing a threat to food availability. This research aims to assess the efficacy of a consortium of five bacteria contained in Mikrobat as bioactivators. The goal is to enhance rice yields, mitigate the severity, and suppress grain rot disease and bacterial leaf blight in rice. The research employed a split-plot design encompassing 12 treatment combinations replicated three times. The experimental setup involved two factors: the main plot consisted of rice varieties-Ciherang, Inpari 4, and Trisakti-while the subplots represented types of bioactivators-Mikrobat, EM4, *Trichoderma asperellum*, and a control. Results demonstrated Mikrobat's positive impact compared to the control, with an average of 17.37 productive tillers and higher yields than other bioactivator treatments. Notably, Mikrobat exhibited 50.04% spikelets per panicle and a 1000-grain weight of 19.77 g. The severity of disease with Mikrobat treatment was notably lower than with EM4 and T. asperellum, featuring 4.20% for grain rot disease and 4.41% for bacterial leaf blight. This was significantly different from the control, which showed severity rates of 9.94% for grain rot disease and 7.75% for bacterial leaf blight. Among the three tested varieties, Ciherang demonstrated the best response, displaying higher resistance to both tested diseases. The varietal treatments did not significantly differ from each other. Although Mikrobat's effects were not significantly distinct from EM4 and T. asperellum, its usage still reduced the severity and suppression of grain rot and leaf blight diseases.

Key words: bioactivator, disease, mikrobat, rice

INTRODUCTION

Rice consumption in Indonesia continues to increase along with population growth, but the production of this commodity has decreased by 0.43% in just a year (BPS, 2021). The decline in rice production cannot be separated from plant diseases, including bacterial grain rot (BGR) caused by *Burkholderia glumae* and bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae*. Yield losses due to *B. glumae* range from 20% to 48% and losses from *X. oryzae* pv. *oryzae* (Xoo) ange from 15% to 24% (Baharuddin et al., 2017; Carsono et al., 2021). These pathogens infect all stages of rice growth, and significant losses due to crop failure can occur if the attack begins in the early stages.

Currently, genetic improvement in resistant varieties is still insufficient to manage the disease because the high adaptability of these pathogens can quickly overcome the resistance of newly introduced varieties. Although rice production in 2022 showed an increase of 1.25 million tonnes (BPS, 2022), this production can still be further increased through the supportive planting of resistant varieties with the integration of proper cultivation techniques and disease control measures. This includes the utilization of beneficial microbes from various groups of bacteria that can colonize and facilitate soil nutrient uptake by plants while suppressing diseases, thereby stimulating plant growth and enhancing production.

Several useful bacteria, namely *Azotobacter* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Streptomyces* sp., and *Paenibacillus polymyxa*, are formulated in liquid form and produced through a biotechnological process. They are patented as "Mikrobat" and have undergone laboratory testing to interact synergistically without antagonizing each other (Baharuddin et al., 2019a). Additionally, all of these bacteria have shown negative outcomes for hypersensitive responses (Sudewi et al., 2022).

The bacteria contained in Mikrobat have been studied for their ability to fix nitrogen, which involves the biological reduction of N_2 into plant-

Corresponding author:
Baharuddin Patandjengi (baharunhas79@gmail.com)

Department of Plant Pest and Disease, Faculty of Agriculture, Hasanuddin University. Jl. Perintis Kemerdekaan No. KM.10 Tamalanrea Indah, Makassar, Indonesia 90245

available NH_4^+ (Herwati et al., 2021). These bacteria can also act as PSB (Phosphate Solubilizing Bacteria) with a high concentration of soluble phosphate, approximately 9000–9382 mg L^{-1} (Sudewi et al., 2020).

In addition to the capabilities of the bacterial consortium, including *Azotobacter* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Streptomyces* sp. and *P. polymyxa*, they have demonstrated the ability to produce IAA and GA in vitro (Faridah et al., 2018). Moreover, these bacteria are capable of producing siderophores, which enables them to inhibit the growth of pathogens and suppress the development of diseases in rice (Sudewi et al., 2022). As a result, this study aims to determine the ability of the consortium of these five bacteria as bioactivators. The objective is to stimulate rice production and mitigate the severity of BGR and BLB diseases.

MATERIALS AND METHODS

Research Site. The research was conducted in the Green House Teaching Farm, Faculty of Agriculture, Hasanuddin University.

Research Design. The research was conducted using a split plot design with rice varieties as the main plot (Ciherang, Inpari 4, and Trisakti) and types of bioactivators as the subplot (Mikrobat, EM4, *T. asperellum,* and control). The treatment was replicated three times. The *T. asperellum* isolate was obtained from the Laboratory Collection of the Department of Plant Pests and Diseases at Hasanuddin University.

Seed Treatment. The rice seeds of the Ciherang, Inpari 4, and Trisakti varieties were soaked using Mikrobat, EM4 and *T. asperellum* at a dose of 10 mL per L^{-1} of water for each treatment. Seeds that were not treated with bioactivators were used as controls. Seed soaking was carried out for 10 hours. The soaked seeds were then drained and wrapped using small pieces of perforated ziplock plastic. Subsequently, the wrapped seeds were stored in a closed location for 2 consecutive 24-hour periods or until the root on the seed began to emerge. Occasional watering was performed if the seeds started to dry out. Once the roots had emerged, seeding was conducted for a period of 10 days.

Cultivation. Seeds from the seedlings were sown in pots filled with a mixture of soil and manure+rice husk compost in a ratio of 2:1. The soil employed in this study was inceptisol soil sourced from an experimental field at Hasanuddin University. Each pot was planted with 3 clusters of rice and appropriately labeled according to the treatment. Watering was carried out twice a day.

Bioactivator Formulation. The doses of Mikrobat and EM4 were 10 mL L^{-1} of water, whereas *T. asperellum* was used at a dose of 10 g L^{-1} of water, with a spore suspension concentration of 1×10^8 cfu mL^{-1}.

Applications of Bioactivator. The application of the bioactivator was conducted 6 times: : first, in the nursery 5 days prior to transferring to the planting medium; second, 3 days before planting in the pot; and then applications to the rice at the ages of 15 days after planting (DAP), 30 DAP, 45 DAP, and 60 DAP. The bioactivator was applied by spraying it on the leaves, around the root area, and on the plant surface, using 10 mL per plant for most applications, and 20 mL per plant for the 45 DAP and 60 DAP applications.

Source of Disease Inoculum. Inoculum *B. glumae* and *X. oryzae* pv. *oryzae* was naturally obtained due to the frequent occurrence (endemic) of these two pathogens in experimental rice cultivated in the Greenhouse at Hasanuddin University.

Productive Tillers. The total number of productive tillers was determined by counting the plants that had developed panicles within all samples in each rice replication during the harvest.

Spikelets Per Panicle. The percentage of spikelets was calculated based on the total number of filled grains within each panicle.

Weight of 1000 Grains. The weight of thousand grains can be utilized to evaluate grain yield (Mansur & Muazam, 2022). This parameter was calculated based on the weight of 1000 filled grains in each treatment and replication, and then measured in grams (g).

Disease Severity. The severity of BGR and BLB disease (%) can be assessed by tallying the number of leaves or panicles displaying symptoms of damage attributed to the respective primary disease, in accordance with the scale of damage or infection category. Disease severity was calculated using the formula employed by Masnilah et al. (2020):

$$DS = \frac{\Sigma(n \times v)}{N \times Z} \times 100\%$$

Ds = Disease severity (%);
n = Number of leaves infected eith a certain category;
v = Scale value of each attack category;
N = Number of leaves observed.
Z = Highest value scale;

The disease severity in each treatment can be interpreted to characterize levels of plant resistance. The levels of resistance to BGR and BLB diseases, as modified by Yudarwati et al. (2020), are as follows: Resistant if the percentage of severity is ≤ 11% of symptoms in terms of the number of seeds per panicle for BGR, and symptoms of leaf area for BLB; Moderately Resistant (11–25%); Moderately Susceptible (25–85%); Susceptible (> 85%).

Data Analysis. The data were analyzed using analysis of variance (ANOVA), followed by post hoc testing with Tukey's Honestly Significant Difference at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The results showed that the bioactivator treatments had a significant effect on the number of productive tillers, spikelets per panicle, and the weight of 1000 grains (Table 1). The application of bioactivators yielded better results in terms of productive tillers compared to the control. Although *T. asperellum* produced high results, they were not significantly different from EM4 (Table 1). The application of bioactivators on rice can lead to an increased number of productive tillers as it helps fulfill the phosphorus (P) nutrient requirements during the reproductive stage. Phosphorus is known to play a role in promoting early flowering (Jiban et al., 2020).

The percentage of spikelets per panicle was notably higher with the Mikrobat treatment compared to the other treatments (Table 1). Mikrobat yielded the highest outcome, with an average percentage of spikelets per panicle at 50.04%. While not displaying significant differences from EM4 and *T. asperellum*, the consortium of bacteria contained in Mikrobat, such as *Azotobacter* sp., *Lactobacillus* sp., *Pseudomonas* sp., and *P. polymyxa*, possesses nitrogen-fixing capabilities (Herwati et al., 2021). This contributes to the enhanced nitrogen uptake rate in rice, resulting in a greater spikelets-per-panicle count in the Mikrobat treatment compared to the others. This presumption is supported by Jiban et al. (2020), who asserted that adequate nitrogen nutrients could optimize rice's yield potential by steadily increasing both panicle numbers and the number of filled spikelets.

Mikrobat exhibited the highest weight of 1000-grain yield in comparison to other treatments, with an average 19.77 g (Table 1). The components of Mikrobat, namely *Azotobacter* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Streptomyces* sp. and *P. polymyxa*, possess the ability to produce indole-3-acetic acid (IAA) and gibberellin (GA), which play crucial roles in various aspects such as promoting growth and development of roots, leaves, flowering, and grain ripening (Faridah et al., 2018; Baharuddin et al., 2019b). However, the thousand-grain weight still fell short of the general average of 25–28 g per cluster (Mansur & Muazam, 2022). Nonetheless, the application of bioactivators resulted in significantly higher yields. The range of thousand-grain weight with bioactivators was 17.16–19.77 g, while the control yielded only 9.01 g. Furthermore, the presence of grain rot disease in the early stages of rice growth also influenced the thousand-grain weight, as infections caused by *B. glumae* could disrupt the grain-filling process in rice (Pedraza et al., 2018).

Regarding BGR disease severity, only the bioactivator treatment yielded significant results. However, in terms of BLB disease severity, a significant interaction between varieties and bioactivators was observed (Table 2, Table 3). The highest BGR severity was identified in the Trisakti variety (Figure 1), indicating its susceptibility to BGR disease. Conversely, the highest BLB severity was noted in the Inpari 4 variety (Figure 2). Sastro et al. (2021) reported that the Inpari 4 variety demonstrated moderate resistance to BLB strains III and IV, while it was moderately susceptible to BLB strain VIII. On the other hand, the Ciherang variety exhibited resistance to BLB strains III and IV. The resistance of rice varieties was linked to the pathotype, as different pathotypes of *X. oryzae* pv. *oryzae* and *B. glumae* displayed varying levels of virulence (Rasmiyana et al., 2019; Jungkhun

Table 1. Effect of bioactivator on number of productive tillers, spikelets per panicle, and weight of 1000 grain of rice

Bioactivators Treatment	Productive tiller	Spikelets per panicle	Weight of 1000 grains (g)
Kontrol	5.44 c	34.46 b	9.01 b
Mikrobat	17.37 b	50.04 a	19.77 a
EM4	17.63 ab	45.96 a	17.16 a
T. asperellum	18.07 a	42.29 ab	19.01 a

Numbers on the same coloum followed by different letters showed a significant difference (HSD; $\alpha = 5\%$).

Tabel 2. Effect of bioactivators on severity of bacterial grain rot (BGR) disease in rice at 90 DAP

Bioactivators	Bacterial grain rot disease severity (%)
Control	9.94 a
Mikrobat	4.20 b
EM4	4.94 b
T. asperellum	4.80 b

Numbers on the same line followed by different letters showed a significant difference (HSD; α = 5%). DAP= day after planting.

Tabel 3. Combination effect of rice varieties and bioactivators on severity of bacterial leaf blight (BLB) disease at 90 DAP

Treatments	Bacterial leaf blight disease severity (%)			
	Control	Mikrobat	EM4	*T. asperellum*
Ciherang	4.50 b	3.67 b	3.98 b	5.27 b
Inpari 4	13.27 a	4.15 b	6.59 b	6.98 b
Trisakti	5.49 b	5.40 b	5.24 b	5.90 b

Numbers followed by different letters showed a significant difference (HSD; α = 5%). DAP= day after planting.

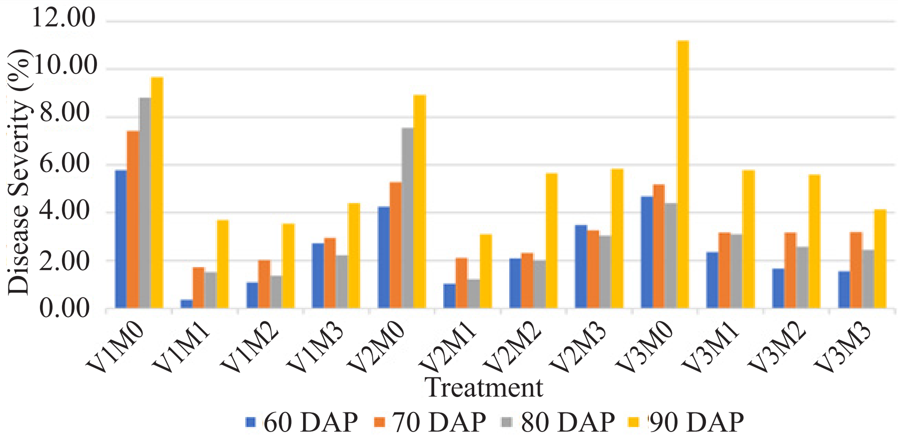

Figure 1. Severity of BGR disease at 60–90 DAP. V1= Ciherang, V2= Inpari 4, V3= Trisakti, M0= control, M1= Mikrobat, M2= EM4, and M3= *T. asperellum*.

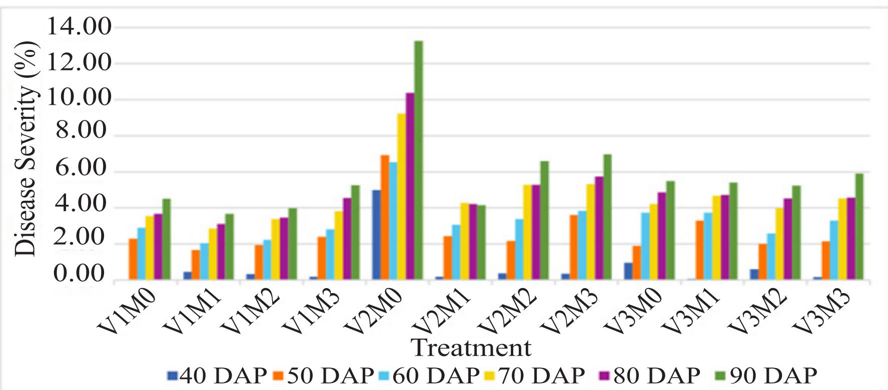

Figure 2. The severity of BLB at 40 DAP–90 DAP. V1= Ciherang, V2= Inpari 4, V3= Trisakti, M0= control, M1= Mikrobat, M2= EM4, and M3= *T. asperellum*.

et al., 2022).

In this research, the disease severity of BGR and BLB was effectively suppressed in the Mikrobat treatment. The bacteria within Mikrobat are capable of producing siderophores as their secondary metabolites, playing a significant role as plant growth-promoting rhizobacteria (Sudewi et al., 2022). As noted by Nabila & Kasiamdari (2021), siderophores can act as virulence factors in various soil pathogens. When bacteria produce siderophores under conditions of low iron availability, pathogens are unable to utilize the resulting ferric-siderophore complex. Siderophore-producing bacteria can exploit this complex through specific receptors on their outer cell membrane, thereby restricting the growth of pathogens (Gupta et al., 2020).

Taking into consideration the comprehensive results obtained from each parameter, it is evident that the utilization of bioactivators has a superior impact compared to the control. Mikrobat exhibits the potential to serve as a bioactivator for stimulating rice growth and production while concurrently suppressing BGR and BLB diseases.

CONCLUSIONS

Using Mikrobat as bioactivators from five bacterial consortium had a positive effect on the parameters of the number of productive tillers (17.37), spikelets per panicle (50.04%) and the weight of 1000 grains (19.77 g). Although not significantly different from the using EM4 and *Trichoderma asperellum*, but using Mikrobat was able to reduce the severity and suppress BGR and BLB at 90 DAP.

ACKNOWLEDGMENTS

The author extends sincere thanks to Direktorat Jenderal Pendidikan Tinggi, Riset dan Teknologi, Kementerian Pendidikan, Kebudayaan, Riset dan Teknologi for their support in funding this publication.

FUNDING

This research was supported by the Direktorat Jenderal Pendidikan Tinggi, Riset dan Teknologi, Kementerian Pendidikan, Kebudayaan, Riset dan Teknologi through the Kedaireka Matching Fund 2022 Program as the funding source, with contract number: 319/E1/KS.06.02/2022.

AUTHORS' CONTRIBUTIONS

BP, TK, and MJ were involved in planning, consideration, and research oversight. FKS and NH were responsible for manuscript preparation. N supervised the experiment. NFAK contributed to data collection in the field. All authors participated in providing responses and comments on the research, including validating data analysis, and have read and approved the final manuscript.

COMPETING INTEREST

This bioactivator formulation will be scaled up and produced by PT Karanta Duta Utama.

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